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Giant Cell Formation in Cells Exposed to 740 nm and 760 nm Optical Traps

Hong Liang, PhD, Ky Trong Vu, Tina Ching Trang, David Shin, Yider Eddie Lee, Ducc Chi Nguyen, Bruce Tromberg, PhD, and Michael W. Berns, PhD*

Beckman Laser Institute and Medical Clinic, University of California, Irvine, California

Background and Objective: **Optical trapping is becoming a useful and widespread technique for the micromanipulation of cells and organelles. Giant cell formation following optical trapping was studied to detect the potential adverse effects.**
Study Design/Materials and Methods: **The nuclei of preselected single CHO cells were exposed to 740 nm and 760 nm laser microbeam generated by a titanium-sapphire tunable laser at 88 and 176 mW and different time exposures. The irradiated single cells were recorded and observed morphologically following exposure. Giant cells were tabulated and photographed.**
Results: **The irradiated cells either failed to divide, or they underwent nuclear proliferation to form giant cells through endoreduplication.**
Conclusion: **Giant cells were induced by both 740 nm and 760 nm. The frequency of giant cell formation was higher for the longer time exposures and at the higher power densities. The use of an optical etalon to remove intracavity mode beating and high peak powers of the titanium-sapphire laser caused a significant reduction in the formation of giant cells. Lasers Surg. Med. 21:159–165, 1997.** **© 1997 Wiley-Liss, Inc.†**

Key words: Chinese hamster ovary (CHO); chromosome; interphase; laser microirradiation; nuclei; titanium-sapphire laser; two-photon absorption

INTRODUCTION

Optical trapping is becoming a useful and widespread tool for the manipulation of cells and organelles. However, there has been little attention given to the potential adverse effects of the trap beam on the biological system being studied. Here, we examine giant cell formation as a result of exposing cell nuclei to laser optical traps.

Giant cell formation has been described in many cell systems and may occur spontaneously or by a variety of physical or chemical experimental treatments [1–8]. It can result from cell fusion or continued growth of single cells under conditions where amitosis or endoreduplication take place. In 1981, Cremer et al.[5] used a pulsed laser microbeam of 532 nm wavelength to produce visible small lesions in the nucleoplasm of V79 Chinese hamster cells. The cells containing nuclear lesions did not enter mitosis but formed giant cells. In a recent study on wavelength dependence of induced chromosome bridges [9], it was found that laser-induced optical trapping of chromosomes in mitotic PTK_2 cells using 740 and 760 nm wavelengths induced abnormalities of chromosome behavior, which included chromosome bridges or c-mitosis, i.e., complete blockage of chromosome separation.

Because of increasing interest and use of laser optical traps in cell biology as well as the pre-

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*Correspondence to: Dr. Michael W. Berns, Beckman Laser Institute and Medical Clinic, University of California at Irvine, 1002 Health Sciences Road East, Irvine, CA 92715.

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vious observations of laser trap-induced abnormalities in chromosome separation in mitosis [10–13], we have undertaken a more detailed analysis of the effects of these trapping wavelengths [14]. In this report, we describe multinucleated giant cell formation in Chinese hamster ovary (CHO) cells using either 740 nm or 760 nm optical trapping beams generated by the Titanium-sapphire laser. In addition, we examine the possibility of multiphoton absorption as a contributing factor to the giant cell formation.

MATERIALS AND METHODS

Cell Culture

Chinese hamster (*Cricetulus griseus*) ovary (CHO) cells obtained from the American Type Culture Collection (CCL no. 61) were used in the experiments. CHO cells are able to grow very well under low cell density conditions. This facilitates a successful follow-up of a laser microirradiated single cell up to 5–6 days until confluence is reached. The cells were maintained in GIBCO's minimum essential medium (MEM) with 10% (vol./vol.) fetal bovine serum (Life Technologies, Grand Island, NY) and were regularly subcultured using 0.25% trypsin (Life Technologies). In preparation for the experiment, the cells were grown in T-25 tissue culture flasks (Corning, Newark, CA), until they reached the desired confluence. The cells were then collected and injected into Rose chambers in the density of 3×10^3 cells/ml, 4–5 hr prior to laser microirradiation [14].

Laser Microbeam Instrumentation

A Titanium-sapphire laser tunable between 700–1000 nm (Model 889, Coherent, Palo Alto, CA) was employed in this study. The laser was directed into a Zeiss photomicroscope and subsequently focused to a 0.5–1.0 μm spot diameter by a Neofluar X100 phase-contrast objective with a numerical aperture of 1.3 (Carl Zeiss, Thornwood, NY). A dichroic mirror deflected the laser beam into the photomicroscope, at the same time allowing the visible light to pass to a video camera. The video image was recorded by a half-inch time lapse VCR (Panasonic Corp., Secaucus, NJ) and displayed on a monochrome monitor.

During all the experiments, a constant temperature at 37°C was maintained using an air-curtain incubator (Model ASI 400, Nicholson Precision Instruments, Bethesda, MD) in the area of the microscope stage where the Rose chamber was placed.

Calibration of Laser Power

To determine the power reaching the irradiated sample, the dual-objective transmittance measuring technique of Misawa et al. [15] was used. In this method, two identical and opposite facing microscope objectives first focused and then recollimated the incident beam into an optical power meter. This method eliminates total internal reflection errors that are encountered in a direct objective-to-power meter measurement in air. In the dual objective method, the transmission through a single microscope objective is then the square root of the measured transmittance. In our experiment, the transmission through a single oil-immersion objective determined from the dual-objective method was 0.58. In comparison, a direct objective-to-power meter measurement in air gave a transmission of 0.33, which is 57% of the true value. Careful consideration should be given to the technique used in various published studies to measure laser irradiance at the focal point.

Laser Microirradiation of Single CHO Cells and Follow-Up

Isolated healthy single cells in interphase were chosen for all experiments. The position of the pre-selected cell was first marked by scribing a small circle around it on the outside coverslip surface of the culture chamber using a Zeiss diamond objective marker. A second larger circle was drawn around the first diamond cut circle using a permanent marker pen. The marker pen circle facilitated rapid visual relocation of the experimental single cell under the microscope during follow-up. The cell density of 3×10^3/ml was diluted enough to keep the experimental single cell adequately isolated from other individual single cells and enclosed within the circumscribed region for at least within 3–4 days. The nucleus of the pre-selected cell was placed under the crosshairs on the monitor screen. The crosshairs denoted the focal point of the optical trap. The laser microirradiation was then initiated at this specific site. The laser trapping power in the objective focal spots was either 88 mW or 176 mW, corresponding to power densities of 3×10^7 W/cm^2 and 6×10^7 W/cm^2, respectively.

After laser microbeam irradiation, the Rose chamber was maintained in a CO^2 incubator at 37°C. The irradiated single cells were followed

and observed morphologically at least four times a day to confirm that no alien cells migrated into the circumscribed region for up to 5 days following exposure. Photography was employed utilizing Kodak Plus-X film (Eastman Kodak Co., Rochester, NJ) and the internal camera of the microscope.

RESULTS

Occurrence and Morphology of Giant Cells

Cells at least twice the size of normal cells are designated as giant cells. Giant cells are large and either multi-nuclear or uninuclear. Figure 1 illustrates the normal control cells derived from a single cell. Figure 2 presents examples of giant cells induced by exposure to the 760 nm trapping laser beam. The cell in Figure 2A is an extremely long-shaped and uninuclear cell. It appeared on the 4th day after 5 seconds in a trap of 88 mW. The cell was alive, but no cell division occurred. In contrast, Figure 2B, C contains examples of multinuclear giant cells at the 5th day following exposure to the laser trap. Micronuclei were also observed in these cells. Figure 2D illustrates two giant cells derived from an irradiated single cells at the 6th day. Giant cell formation also occurred at 740 nm laser microirradiation. Large multinuclear or uninuclear cells were frequently induced (Fig. 3). The giant cells were morphologically similar to those formed following the 760 nm treatment.

The frequencies of giant cell induction by 760 nm and 740 nm at 88 and 176 mW for different durations of exposure are presented in Table 1.

Giant cells do occur spontaneously; 411 isolated nontreated control single cells at interphase were randomly selected and followed. Only four cells (1%) became giant cells.

Giant Cell Formation by 760 nm Laser Microirradiation Due to Possible Multiphoton Events

Preliminary observations indicated that at certain wavelengths random longitudinal mode beating occurs in the laser cavity resulting in moderately higher peak-power pulses [16]. When the laser beam is focused to a diffraction-limited spot, sufficiently high photon densities in the focal spot could induce multiphoton absorption and subsequent adverse cellular effects comparable to single photon absorption in the UVA (320–400 nm) region of the spectrum. This longitudinal

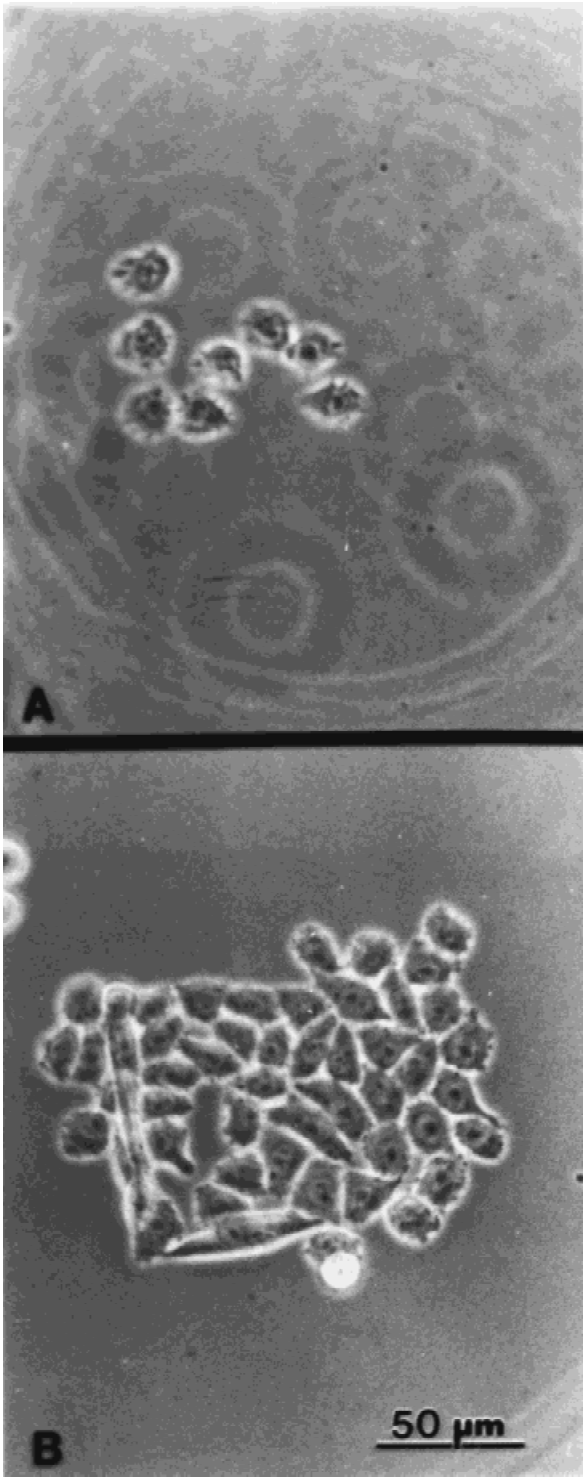

Fig. 1. Control cells with normal clonal growth derived from a single unirradiated cell: **A,** 8 cells, the 3th day; **B,** > 50 cells, the 5th day.

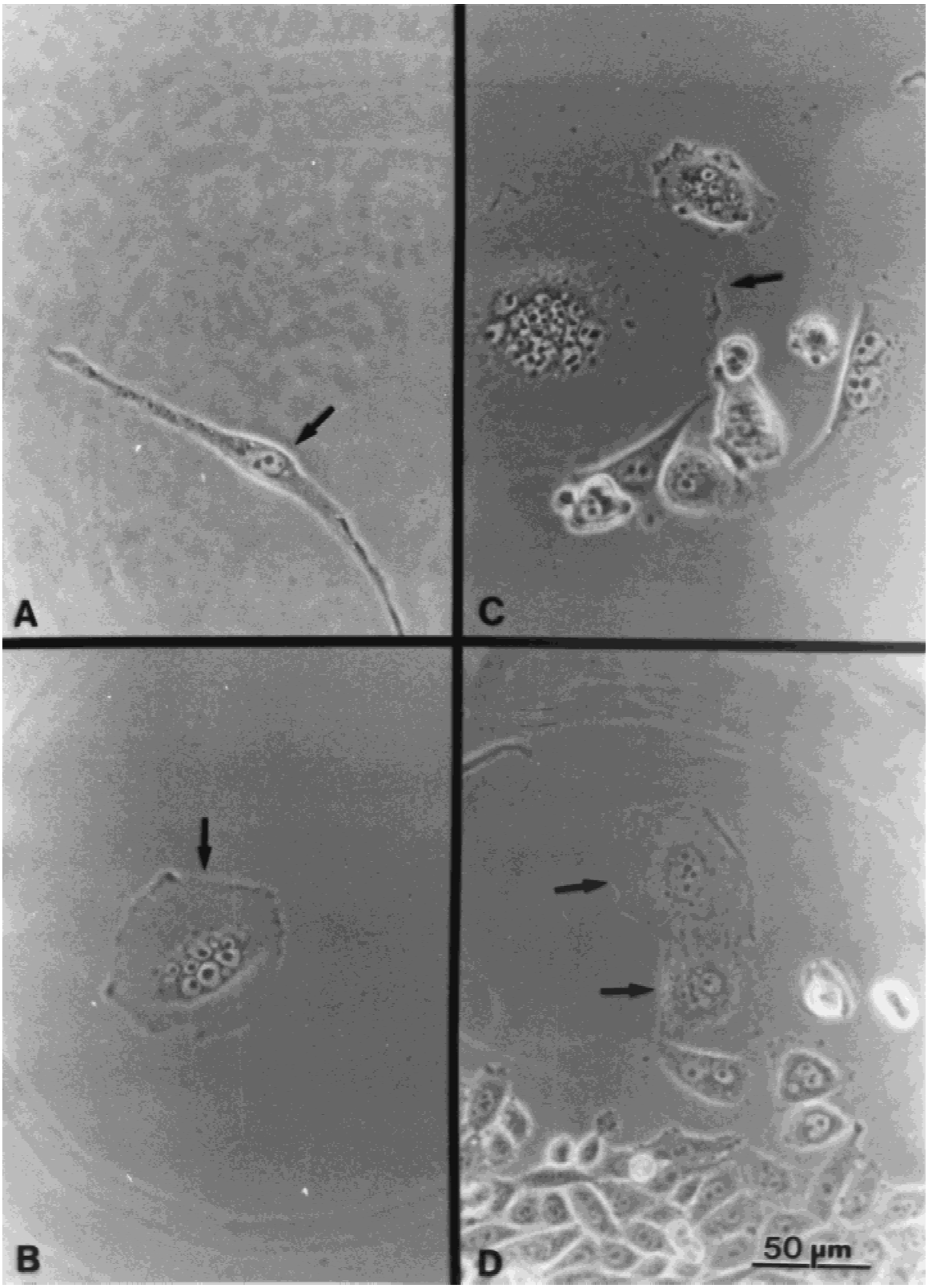

Fig. 2. Examples of induced giant cells after nuclear microirradiation at 760 nm: **A,** long-shaped uninuclear cell, **B,** a multinuclear giant cell, **C,** a multinuclear giant cell, **D,** two giant cells from an irradiated cell.

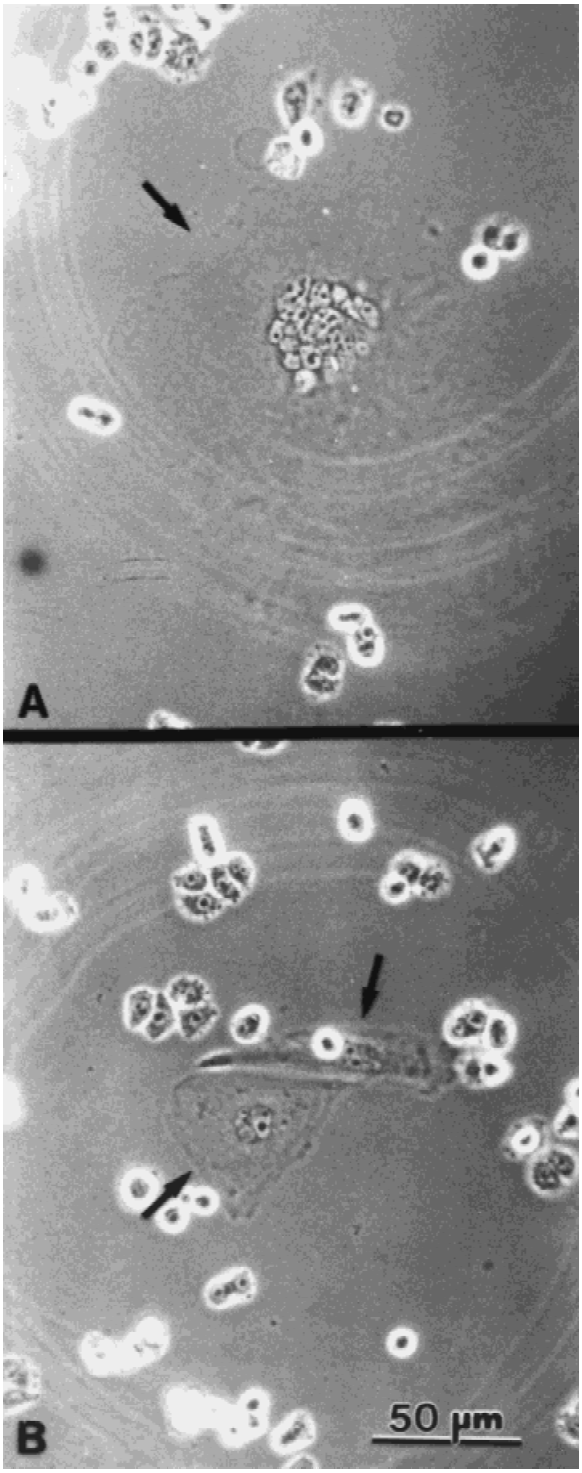

Fig. 3. Examples of giant cells after nuclear microirradiation at 740 nm: **A,** a multinuclear giant cell, **B,** two giant cells from an irradiated cell.

TABLE 1. Induction Frequency of Giant Cells by 740 and 760 nm Laser Microirradiation of Nuclei

Wavelength (nm)	Power (mW)	Exposure time (sec)	Energy density (J)[a]	No. of cells studied	% of giant cells
740	88	20	60	15	0
		60	180	15	33.3
		180	540	15	40.0
		300	900	32	34.5
	176	5	30	10	0
		10	60	10	0
		20	120	10	30.0
		60	360	10	20.0
		180	1080	10	0
760	88	1	3	20	0
		5	15	38	36.8
		10	30	37	29.6
		20	60	12	33.3
	176	1	6	10	20.0
		3	18	10	60.0
		5	30	20	30.0

[a]Unit: 10^7 J/cm^2.

mode beating was eliminated by the use of an intracavity etalon. The etalon-modified laser output was at a single frequency (20-MHz linewidth). In a previous study, two photon excited fluorescence using 760 nm was demonstrated in optically trapped sperm cells stained with Rhodamine 123. In addition, the cell damage evaluated by cloning efficiency in CHO cells demonstrated that more cell death was induced using multimode exposure as compared to etalon modified single frequency exposure [17]. This result is relevant to the giant cell formation reported here. The results of giant cell formation with and without the use of the etalon are summarized in Figure 4. It is clear that the use of the etalon reduces the number of giant cells formed at the shorter time exposures.

DISCUSSION

In 1981, Cremer et al. [5] used a pulsed laser microbeam of 532 nm to produce visible small lesions in the nucleoplasm of V79 Chinese hamster cells. The cells containing nuclear lesions were unable to undergo mitosis and instead formed giant cells. Giant cell formation could be induced in cells with a nuclear lesion produced at a random chromosome site. Other evidence of giant cell formation was obtained by microirradiation of chromosomes during mitosis. In a recent study on the wavelength-dependence of chromosome bridge induction [9], it was found that laser microirradiation of chromosomes in mitotic PTK_2 cells at 760 nm produced abnormalities of chromosome be-

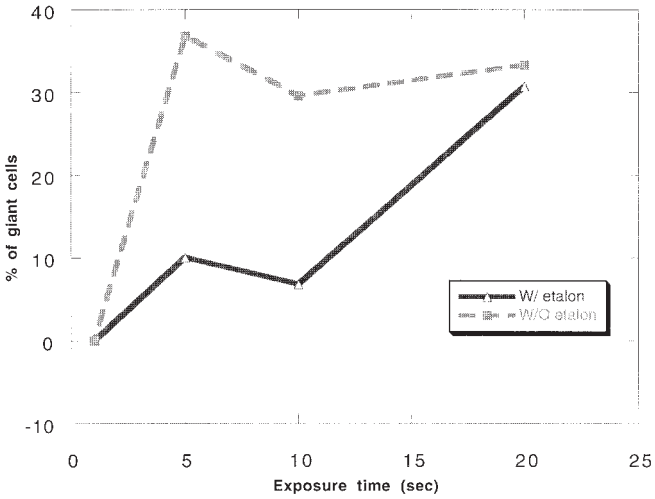

Fig. 4. Comparison of giant cell formation in CHO cells after trapped in a 760 nm laser microbeam at 88 mW with and without etalon.

havior in 100% of the cells irradiated. This included chromosome bridges or c-mitosis, i.e., complete blockage of chromosome separation. Previously, it was demonstrated that maximum cell damage occurred at 740–760 nm [14]. Trap beams < 800 nm are most likely capable of two photon excitation of endogenous cellular absorbers, such as molecules of the respiratory chain (reduced pyridine co-enzymes) [16,17]. These molecules and other endogenous chromophores wherein excited electronic states, can produce cytotoxic oxygen radicals and singlet oxygen, which result in cell damage [18,19].

In the present study, we make the following conclusions about the effects of 740 and 760 nm optical trapping wavelengths on giant cell formation: (1) giant cells are induced by both 740 nm and 760 nm optical trapping wavelengths, (2) giant cells are more readily induced by 760 nm than 740 nm, (3) the frequency of giant cell formation is higher for the longer time exposures (total light dose) and higher power densities. However, at the higher light dose and power density, all the cells are killed, thus no giant cell formation is detected, and (4) the use of an optical etalon to remove the intracavity mode beating and consequent production of higher peak powers at 760 nm causes a significant reduction in the formation of giant cells. However, after 20 seconds of laser exposure both the etalon and nonetalon groups exhibited an equal percentage of giant cell formation.

The results of the etalon experiments strongly suggest that two photon absorption occurs and can induce giant cells. The fact that there is no difference between both groups at the longer exposures (total doses) is unexpected. However, it is possible that giant cell formation is affected by two photon and one photon absorption. With longer exposure times, the accumulation of single photon effects in the etalon group would eventually equal the effect of the two photon-induced events. However, another possibility would be that two photon absorption occurs at a much lower rate in the etalon group. It would, therefore, take longer exposure times to accumulate the same number of effects. Recent studies demonstrating two photon-induced fluorescence from this laser beam system even with the etalon in place support this hypothesis [16,17].

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REFERENCES

1. Hetzel FW, Kolodny GM. Radiation-induced giant cell formation: The influence of conditions which enhance repair of potentially lethal damage. Radiat Res 1976; 68: 490–498.
2. Wang N-S, Seemayer TA, Ahmed MN, Knaaek J. Giant cell carcinoma of the lung, a light and electron microscopic study. Human Pathol 1976; 7:3–16.
3. Nakanishi K, Fujita S. Molecular mechanism of polyploidization and binucleate formation of the hepatocyte. Cell Struct Funct 1977; 2:261–265.
4. Geisinger KR, Leighton J, Zealberg J. Appearance of giant cells as a morphological response of rat bladder carcinoma cell line (Nara bladder tumor no. 2) to a continuous thermal gradient in tissue culture. Cancer Res 1978; 38:1223–1236.
5. Cremer T, Turner A, Liaw L-HL, Berns MW. Giant cell formation produced by laser microbeam irradiation of chromatin in Chinese hamster cells. Exp Cell Res 1981; 134:49–63.
6. Kanai-Azuma M, Kanai Y, Kurohmaru M, Sakai S, Hayashi Y. Insulin-like growth factor (IGF)-I stimulates proliferation and migration of mouse ectoplacental cone cells, while IGF-II transforms them into trophblastic giant cells in vitro. Biology of Reproduction 1993; 48:252–261.
7. Bird MC, Carside D, Jones, HB. Multinucleated giant cells in primary culture derived from canine bone marrow—evidence for formation of putative osteoclasts. Cell and Tissue Research 1992; 268:17–30.
8. Enelow RI, Sullivan GW, Carper HT, Mandell GL. Induction of multinucleated giant cell formation from in vitro culture of human monocytes with interleukin-3 and in-

terferon-gamma: Comparison with other stimulating factors. Am J Respiratory Cell Molec Biol 1992; 6:57–62.
9. Vorobjev IA, Liang H, Wright WH, Berns MW. Optical trapping for chromosome manipulation: A wavelength dependence of induced chromosome bridges. Biophys J 1993; 64:533–538.
10. Berns MW, Wright WH, Tromberg BJ, Profeta GA, Andrews JJ, Walter RJ. Use of a laser-induced optical force trap to study chromosome movement on the mitotic spindle. Proc Natl Acad Sci USA 1989; 86:4539–4543.
11. Berns MW, Aist JR, Wright WH, Liang H. Optical trapping in animal and fungal cells using a tunable near-infrared titanium-sapphire laser. Exp Cell Res 1992; 198: 375–378.
12. Liang H, Wright WH, He W, Berns MW. Micromanipulation of mitotic chromosome in PTK_2 cells using laser-induced optical forces (Optical tweezers) Exp Cell Res 1991; 195:21–35.
13. Liang H, Wright WH, Cheng S, He W, Berns MW. Micromanipulation of chromosomes in PTK_2 cells using laser microsurgery (optical scalpel) in combination with laser induced optical force (optical tweezers). Exp Cell Res 1993; 204:110–120.
14. Liang H, Vu KT, Krishnan P, Trang TC, Shin D, Kimel S, Berns MW. Wavelength dependence of cell cloning efficiency after optical trapping. Biophys J 1996; 70:1529–1533.
15. Misawa H, Koshioka M, Sasaki KK, Kitamura N, Masuhara H. Three dimensional optical trapping and laser ablation of a single polymer latex particle in water. J Appl Phys 1991; 70:3829–3836.
16. Konig K, Liang H, Berns MW, Tromberg, BJ. Cell damage by near-IR microbeams. Nature 1995; 377:20–21.
17. Konig K, Liang H, Berns MW, Tromberg, BJ. Cell damage in near-infrared multimode optical traps as a result of multiphoton absorption. Optic Letters 1996; 21:1090–1092.
18. Cunningham ML, Jhonson JS, Giovanazzi SM, Peak MJ. Photosensitized production of superoxide anion by monochromatic (290–405 nm) ultraviolet irradiation of NADH and NADPH coenzymes. Photochem Photobiol 1985; 42: 125–128.
19. Tyrrell RM, Keyse SM. New trends in photobiology (Invited review) the interaction on UVA radiation with cultured cells. J Photochem Photobiol B Biol 1990; 4:349–361.